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Suminar E · Rahmawati V · Sumadi · Nuraini A

In vitro multiplication of strawberry in various types and concentrations of cytokinins and auxins

Abstract. Growth regulator is one of the important factors in the success of plant propagation by tissue culture. 6-Benzylaminopurine (BAP) is one of the most widely used cytokinin because it is more stable, less expensive and more effective. Thidiazuron (TDZ) can increase the action of other cytokinins, either exogenous cytokinin or endogenous cytokinin. 1-Naphthaleneacetic acid (NAA) belongs to the more stable auxin group, because it is not easily decomposed and oxidized by enzymes released by plants. This study aimed to obtain the best types and concentrations of BAP, TDZ, and NAA for the *in vitro* multiplication of strawberry explant. The experiment was conducted at the Seed Technology and Tissue Culture Laboratory, Faculty of Agriculture, Universitas Padjadjaran, from April to July 2022. The explants used were axillary shoots of strawberry var. Sweet Charlie is derived from plantlets that were propagated *in vitro*. The experimental design used was a completely randomized design with six treatments and four replications. The media used were Murashige dan Skoog (MS) with the addition of plant growth regulators (PGRs) in the form of BAP (0 ppm and 0,5 ppm), TDZ (0 ppm and 0,1 ppm), and NAA (0 ppm and 0,1 ppm). The application of different types and concentrations of BAP, TDZ, and NAA resulted in different effects of *in vitro* shoot multiplication of strawberry explant var. Sweet Charlie. Concentration of 0.1 ppm TDZ produced the highest number of shoots (5.38).

Keywords: BAP • NAA • Strawberry • TDZ • Tissue culture

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Suminar E[1] * · Rahmawati V[2] · Sumadi[1] · Nuraini A[1]
[1]Department of Agronomy, Faculty of Agriculture, Universitas Padjadjaran. Jalan Raya Bandung Sumedang km 21, Jatinangor, Sumedang 45363 Indonesia.
[2]Undergraduate Program of Agrotechnology, Faculty of Agriculture, Universitas Padjadjaran. Jalan Raya Bandung Sumedang km 21, Jatinangor, Sumedang 45363 Indonesia.
*Correspondence: erni.suminar@unpad.ac.id.

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Introduction

Strawberry (*Fragaria x ananassa*) is one of the fruit plants with high economic value and greatly demanded by people around the world. It has high nutritional and antioxidant content because its quercetin, ellagic acid, anthocyanins, and kaempferol content (Ministry of Agriculture of the Republic of Indonesia, 2019). Cultivated strawberries in Indonesia are frequently resulted from introductions, i.e., cv. Sweet Charlie from the United States of America (USA).

The demand for strawberries in Indonesia is quite high, but it is not accompanied by the level of production. Production of strawberries in Indonesia continues to decline. Strawberry's production in 2020 was 8.350 tons, a decrease from 2017 at 12,225 tons and 2018 at 8,531 tons (Statistics Indonesia, 2020).

Strawberry plants are susceptible to disease caused by viruses that infect plants, this is one of the main factors causing a decrease in strawberry yields of up to 80% (Research Institute for Citrus and Subtropical Fruit Crops, 2015; Thompson & Jeikman, 2003). Apart from that, powdery mildew fungus infection can reduce the productivity of strawberry plants in producing fruit (Wahyuni, 2016). The system for procuring strawberry seeds with conventional methods, both generative and vegetative (with stolons), is still less effective in producing superior and disease-free seeds because it has the potential to transmit congenital diseases from parental plants (Widiastuti, 2015).

One of the methods to provide strawberry seeds in large quantities that are free of disease is by tissue culture technique. The tissue culture technique has several advantages compared to other conventional techniques, such as being able to produce seeds on a large scale, being uniform, healthy, or free of pathogens, and not requiring large areas of propagation land (Hardiyati et al., 2017).

The shoot multiplication stage is one of the important stages in tissue culture technique. The addition of PGRs to the culture medium can improve plant multiplication ability in tissue culture (Wahyuni et al., 2020). According to Lestari (2011), PGRs are regularly used in the culture medium in tissue culture are types of auxins and cytokinins whose use depends on the purpose or direction of the desired plant growth. Comparison of the appropriate concentrations of PGRs of auxin and cytokinin in tissue culture is known to stimulate a faster shoot multiplication process.

Bimantara (2018) who work with strawberry cv Earlibirte meristem culture, showed that the treatment of 0.5 ppm BAP + 0.025 ppm NAA gave the best results on the number of shoots and number of leaves. The addition of 0.50 ppm BAP has a good effect on the number of shoots, number of leaves, plantlet fresh weight, and runner, while the excessive use of TDZ gave defective results on the growth of shoots, leaves, and roots of strawberry plantlets cv Tochiotome (Raisya et al., 2020).

The addition of cytokinins in the form of BAP or TDZ which plays a role in stimulating shoot growth and cell division, as well as auxin in the form of NAA which plays a role in stimulating root growth and cell elongation, are expected to have a positive effect to improve strawberry multiplication ability. The information about the role of BAP, TDZ, and NAA in the *in vitro* shoot multiplication stage in strawberry is still not widely known, therefore it is necessary to research the type and concentration of cytokinins (BAP and TDZ) and auxin (NAA), for the *in vitro* shoot multiplication of strawberry explants var Sweet Charlie.

Materials and Methods

The research was conducted from April to July 2022 at the Seed Technology and Tissue Culture Laboratory, Faculty of Agriculture, Universitas Padjadjaran. The tools used in this study include culture bottles, tweezers, scalpels, Laminar Air Flow (LAF), petri dishes, etc. The plant material used in the present experiment was explant in the form of axillary shoots aged 23 days after planting (DAP) from the 5th subculture of the strawberry var Sweet Charlie plantlet that was propagated *in vitro*. Other materials used were BAP, TDZ, and NAA.

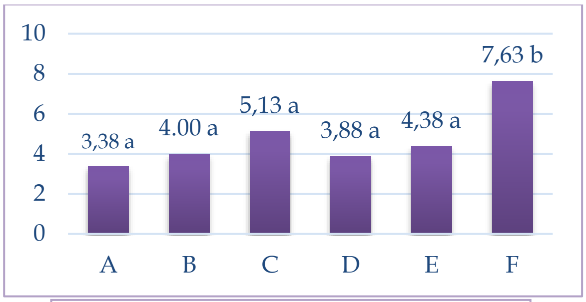
The study was designed with a completely randomized design consisting of six treatments, repeated four times. The multiplication medium was MS base media with 6 treatments of PGR addition, i.e., A (Control/without PGR); B (BAP 0.5 ppm); C (TDZ 0.1 ppm); D (0.1 ppm NAA), E (0.1 ppm NAA + 0.5 ppm BAP); and F (0.1 ppm NAA + 0.1 ppm TDZ).

Observational parameters include shooting emergence time (DAP), number of shoots, number of roots, and fresh weight of plantlets.

Obtained data was analyzed by analysis of variance at 5% and If there is a significant difference, it will continue by the Tukey test. All statistical analysis was performed in SPSS 28.0 software.

Results and Discussion

Shooting Emergence Time. The results of the analysis of variance showed that the addition of cytokinins and auxin had a significant effect on the average time of shoot emergence. According to Kurnia (2014), the addition of exogenous hormones can cause stimulation similar to phytohormones that are naturally produced by plants, so they could affect plant growth and development in the process of cell division, enlargement, and differentiation.



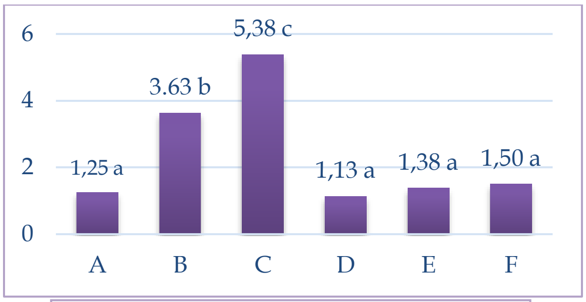
A (Control/without PGRs); B (BAP 0.5 ppm); C (TDZ 0.1 ppm); D (NAA 0.1 ppm), E (NAA 0.1 ppm + BAP 0.5 ppm); and F (NAA 0.1 ppm + TDZ 0.1

Figure 1. Shooting emergence time (DAP)

The experimental results showed that the shoot emergence time in treatment F (0.1 ppm NAA + 0.1 ppm TDZ) was significantly different from treatment A (Control), B (0.5 ppm BAP), C (0.1 ppm TDZ), D (0.1 ppm NAA), and E (0.1 ppm NAA + 0.5 ppm BAP).

Treatment F (0.1 ppm NAA + 0.1 ppm TDZ) gave the highest average shoot emergence time (7.63) compared to other treatments (Figure 1). The addition of exogenous auxins such as NAA is known to stimulate the production of ethylene hormone, especially in high concentrations. The addition of TDZ singly or in combination can inhibit the emergence of shoots, Sjahril et al. (2016) assumed that TDZ with a certain concentration can produce a compound in explants that can slow down the time of shoot emergence. Guo et al. (2011) further explained that TDZ can stimulate the production of the ethylene hormone. According to Zulkarnain (2009), the ethylene hormone in tissue culture can inhibit the process of morphogenesis and shoot growth, so the addition of TDZ either singly or in combination will inhibit the emergence of shoots. TDZ is also involved in the process of auxin synthesis by increasing levels of endogenous auxin, namely indole-3-acetate (IAA) (Cappelletti et al., 2016), so it can be assumed that treatment F (0.1 ppm NAA + 0.1 ppm TDZ) induces more ethylene hormone than other treatments, so it can inhibit shoot formation and result in a longer shoot time.

Number of Shoots. The results of the analysis of variance showed that the addition of PGRs had a significant effect on the average number of shoots at 12 WAP.



A (Control/without PGRs); B (BAP 0.5 ppm); C (TDZ 0.1 ppm); D (NAA 0.1 ppm), E (NAA 0.1 ppm + BAP 0.5 ppm); and F (NAA 0.1 ppm + TDZ 0.1

Figure 2. Number of shoots at 12 WAP

Based on Figure 2, treatments A (Control), D (0.1 ppm NAA), E (0.1 ppm NAA + 0.5 ppm BAP), and F (0.1 ppm NAA + 0.1 ppm TDZ) produced shoots that were not significantly different, but significantly different from treatments B (0.5 ppm BAP) and C (0.1 ppm NAA) at 12 WAP. The average number of shoots in treatment C (0.1 ppm TDZ) was significantly different from treatment B (0.5 ppm BAP), this was presumably due to the addition of 0.1 ppm TDZ can stimulate shoot growth faster than the addition of 0.5 ppm BAP.

The addition of TDZ in low concentrations (0-0.50 ppm) can stimulate the growth of axillary shoots of the strawberry cv Tochiotome, while at high concentrations (more than 0.50 ppm) for a long time can cause abnormality to the explant tissue thereby reducing the ability of shoot regeneration (Raisya et al., 2020), so that it can cause a decrease in shoot micropropagation and inhibit shoot elongation. In the study of

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Cappelletti et al. (2016), it is known that the addition of 0.50 ppm TDZ produced the highest number of shoots (3.60) with an indirect organogenesis process in blueberry cv Duke, while in the research of Raisya et al. (2020), the addition of 0.25 ppm TDZ resulted in the lowest number of shoots (1.70) in strawberry cv Tochiotome.

In this study was found that treatment C with the addition of 0.1 ppm TDZ (Figure 2) resulted in the highest average number of shoots (5.38) at 12 WAP. According to Kusmianto (2008), TDZ can stimulate the production of endogenous cytokinins, and can also act as an inhibitor of cytokinin oxidase which is an enzyme that can eliminate the activity of free adenine-type cytokinins. Therefore, it can be assumed that the addition of 0.1 ppm TDZ singly can increase the production and activity of endogenous cytokinins to induce shoot growth to produce more plantlet shoots.

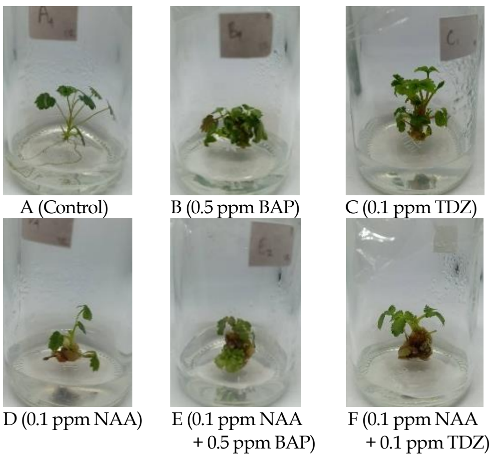
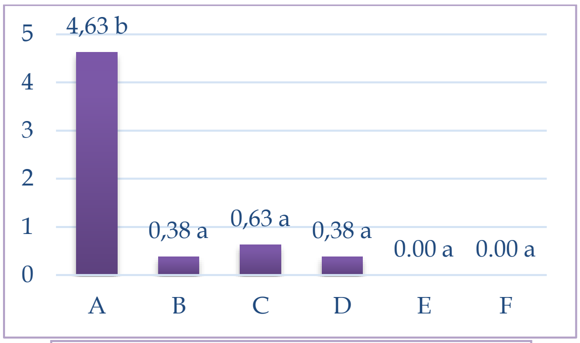


Figure 1. Plantlet form at 12 WAP

Treatment D (0.1 ppm NAA) produced the lowest number of shoots (1.13), but it was not significantly different from treatments A (Control), E (0.1 ppm NAA + 0.5 ppm BAP), and F (0.1 ppm NAA + 0.1 ppm TDZ) at 12 WAP (Figure 2). The low value of the average number of shoots was thought to be due to the addition of PGR in the form of auxin (NAA), which was less effective in promoting the growth of strawberry plantlet shoots. This is in accordance with the research of Sjahril et al. (2016), that the addition of NAA was less effective in stimulating shoot growth and only produced 2.00 shoots with the addition of 0.5 ppm NAA, while the addition of 0.1 ppm and 2.0 ppm NAA did not produce plantlet shoots at all. This was presumably that the addition of auxin to the culture medium can inhibit the growth of axillary shoots in explants (Sjahril et al., 2016).

Strawberry plantlets growing at 12 WAP in treatments B (0.5 ppm BAP) and C (0.1 ppm TDZ) looked denser because they formed a rosette (Figure 3), this was due to a large number of branches and leaves growing on the explants. Rantau et al. (2021) explained that the active growth of new shoots could inhibit the growth of shoot height, causing thicker stems and a more rosette shape of explants. This is presumably that the addition of BAP or TDZ singly in the medium can result in nutritional competition for explants in culture medium due to the higher number of branches and leaves of explants (Danial et al., 2016).

Number of Roots. Based on the results of the analysis of variance, it was found that the PGRs treatment had a significant effect on the average number of plantlet roots of strawberry plants at the end of the observation time (12 WAP). The addition of BAP, TDZ, and NAA resulted in few, short, and no roots.



A (Control/without PGRs); B (BAP 0.5 ppm); C (TDZ 0.1 ppm); D (NAA 0.1 ppm), E (NAA 0.1 ppm + BAP 0.5 ppm); and F (NAA 0.1 ppm + TDZ 0.1

Figure 4. Number of roots at 12 WAP

The number of roots in treatments B (0.5 ppm BAP), C (0.1 ppm TDZ), D (0.1 ppm NAA), E (0.1 ppm NAA + 0.5 ppm BAP), and F (0.1 ppm NAA + 0.1 ppm TDZ) was not significantly different, but significantly different from treatment A (Control). Strawberry explants were grown on medium with the addition of cytokinins and auxins only produced small amounts of roots and did not even produce roots. Rantau et al. (2021) explained that plant explants were less able to form roots in culture medium with the addition of cytokinins and auxins because the addition of exogenous cytokinins and auxins could inhibit the biosynthesis process of endogenous auxin in

forming roots in plant explants. Treatment A (Control) produced the highest number of roots, which was 4.63 (Figure 4). This is in accordance with the research results of Raisya et al. (2020), that the highest number of roots was found in treatment A (without PGRs) which was 1.20 at 4 WAP and 2.70 at 8 WAP.

Plantlet roots were able to grow even though the culture medium was not given additional PGRs in the control treatment, it was presumably that the endogenous auxin content in the explants was quite high so that it could stimulate the formation of plantlet roots even though no PGRs was added (Murti et al., 2012; Haddadi et al., 2010). Plants can naturally produce the auxin hormone even though it is produced in small amounts (Mutmainah, 2016). The addition of a combination of auxin and cytokinin in treatments E (0.1 ppm NAA + 0.5 ppm BAP) and F (0.1 ppm NAA + 0.1 ppm TDZ) was presumably to inhibit root growth but stimulate callus growth due to hormonal balance in the culture.



A (Control/without PGRs); B (BAP 0.5 ppm); C (TDZ 0.1 ppm); D (NAA 0.1 ppm), E (NAA 0.1 ppm + BAP 0.5 ppm); and F (NAA 0.1 ppm + TDZ 0.1

Figure 5. Fresh weight of plantlets (g)

Fresh Weight of Plantlets. The results of the analysis of variance showed that the addition of PGR to the culture medium had a significant effect on the average number of plantlet fresh weight (Figure 5). This proves that the explants can absorb the nutrients contained in the culture medium. Nutrients that have been absorbed by explants are used in the growth process, such as to form shoots, leaves, and roots. The yield of plantlet fresh weight depends on the speed of cell division, cell multiplication, and shoot enlargement (Sulasiah et al., 2015).

Based on Figure 5, it is known that the average number of fresh weight in treatments B (0.5 ppm BAP), C (0.1 ppm TDZ), and F (0.1 ppm NAA + 0.1 ppm TDZ) did not significantly different from treatment E (0.1 ppm NAA + 0.5 ppm BAP), but significantly different from treatments A (Control) and D (0.1 ppm NAA). The highest average yield of plantlet fresh weight (0.51 g) was found in treatment C (0.1 ppm TDZ), although it was not significantly different from treatments B (0.5 ppm BAP), E (0.1 ppm NAA + 0.5 ppm BAP), and F (0.1 ppm NAA + 0.1 ppm TDZ). This is in accordance with the research by Raisya et al. (2020), that the addition of TDZ on culture medium resulted in a large fresh weight of strawberry plantlets (0.50 g), but it was not significantly different from the addition of BAP. This is thought to occur because the addition of TDZ can affect the level of accumulation of minerals or other metabolites (such as amino acids, nucleotides, sugars, lipids, and other compounds) which are relatively high in plant tissues (Angin et al., 2019). That is the response of plant explants to stress and the effects of TDZ to produce thicker shoots (Raisya et al., 2020), so it can affect the yield of plantlet fresh weight.

Conclusions

Based on the results of the study, it can be concluded that:

1. Different types and concentrations of BAP, TDZ, and NAA produced different effects on *in vitro* shoot multiplication of strawberry var Sweet Charlie explants.
2. The addition of 0.1 ppm TDZ resulted in the highest number of strawberry plantlet shoots (5.38).

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